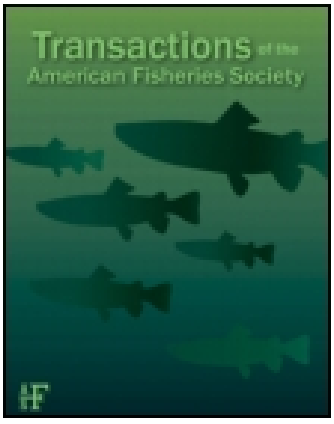

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[Article]

Influence of Rearing Temperature and Feeding Regime on Otolith Increment Deposition in Larval Ciscoes

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Abstract.—Larval ciscoes *Coregonus artedi* were reared in the laboratory from eggs collected in Lake Superior to validate the use of otolith microstructure for estimating age in days. Throughout a 75-d period, water temperatures in two laboratory streams were increased progressively (from 5.6°C to 7.0°C and from 6.1°C to 12.3°C), mimicking the range larvae would probably experience in Lake Superior. Otolith increments were deposited at a rate not statistically different from one increment per day, starting from approximately 28 d posthatch, which was near the time of complete yolk sac absorption. Feeding frequency (twice daily, once daily, alternate days, and food deprivation) did not affect the prominence of subdaily marks (i.e., bands occurring in between daily increments), but a 5-d period of food deprivation reduced increment contrast in some larvae. The reduced rations partly explained lower growth rates among larvae, and larvae with lower growth rates could not be aged as accurately by otolith analysis. An accessory check mark was induced by a sudden decrease in temperature but not by a reciprocal increase in temperature (between 6.9°C and 12.3°C). These results indicate the potential use of otolith analysis for aging larval ciscoes but are strongly cautionary about the method's limits at reduced growth rates. The results also suggest that patterns in otolith microstructure can be used to track shifts in environmental conditions, particularly temperature and feeding success, which may influence growth and survival of ciscoes during early life.

The cisco *Coregonus artedi* was historically the most commercially productive fish species in the Great Lakes until overexploitation led to its decline beginning in the early 1900s (Smith 1968, 1972; Fleischer 1992). At present, substantial stocks of cisco persist only in Lake Superior, the St. Mary's River, and northern Lake Huron, including Georgian Bay (Fleischer 1992). In Lake Superior, recovery to pre-exploitation levels has been inhibited by highly variable recruitment (Bronte et al. 2003), emphasizing the need for a better understanding of early life ecology. Daily aging, made possible by otolith microstructural analysis (Pannella 1971), has the potential to greatly benefit studies of early life growth and survival of ciscoes and thus to further the understanding of cisco recruitment dynamics.

The examination of otolith microstructures has already improved estimates of larval fish age and growth in many species (Jones 1986). Estimated age and growth can then be further utilized to study recruitment dynamics, mortality, and the timing of life history transitions (Brothers 1981; Campana and Neilson 1985), as well as to locate spawning and nursery locations and migratory routes (Brothers 1981; Jones 2002). Despite the increased use of otoliths for aging and back-calculating the growth of larval fishes (Jones 1992), otolith analysis still requires validation because of interspecific variation and interpretation error (Beamish and McFarlane 1983). Such validation has yet to be performed for the cisco.

Otoliths, the inner ear stones used in hearing and balance, probably originate as calcified primordia excreted by inner ear cells (Campana and Neilson 1985). Otoliths then grow by differential deposition of calcium carbonate (aragonite structure), which forms the broad, translucent incremental zones, and the protein otolin, which forms the narrow, opaque discontinuous zones (Campana and Neilson 1985; Jones 2002). It has been hypothesized that the daily deposition of both zones as a growth increment is driven by an endogenous circadian rhythm entrained by photoperiod (Campana and Neilson 1985). However, short-term temperature fluctuations (Brothers 1981) or feeding periodicity (Neilson and Geen 1982) may mask this regular pattern with subdaily increments. Otoliths may also be characterized by distinctly prominent bands, called check marks, that probably signify periods of stress (Campana and Neilson 1985) (e.g., from hatching, yolk sac absorption, or metamorphosis; Jones 2002). In addition, the commencement of daily increment deposition varies among species, commonly occurring before hatching, at hatching, or shortly after hatching, particularly at yolk sac absorption or first feeding (Jones 1986). This interspecific variation underscores the importance of validation

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despite the seemingly universal phenomenon of daily increments.

Such variation in increment deposition also exists among congeners of the cisco. Rice et al. (1985) found that daily increments for bloaters *C. hoyi* from Lake Michigan began at first feeding, while Eckmann and Rey (1987) found that the European whitefish *C. lavaretus* and sandfelchen *C. fera* from Lake Constance, Germany, exhibited daily deposition after hatch. In contrast, Huuskonen and Karjalainen (1995) could not confirm that deposition was daily for vendace *C. albula* and European whitefish from Lake Pyhäselkä, Finland, possibly because of low calcium concentrations in Finnish lakes (Huuskonen et al. 1998). A low growth rate induced by limited food rations (Rice et al. 1985) or low water temperatures (Klink and Eckmann 1992) can also obscure daily deposition in coregonids.

In this study, the otoliths of laboratory-reared cisco larvae were examined to validate the use of otolith microstructure for daily aging. In particular, our objectives were to determine (1) whether increments are formed daily; (2) whether daily increments commence with a check mark at hatching, first feeding, or at yolk sac absorption; and (3) whether temperature, feeding periodicity, or sudden temperature shifts can affect increment deposition or induce formation of check marks.

Methods

Egg collection and rearing protocol.—Larval ciscoes were reared in the laboratory from eggs. Spawning ciscoes were caught with a gill net from Lake Superior, off the eastern coast of the Keweenaw Peninsula, Michigan, in December 2001. Eggs were squeezed from three ripe females and fertilized with the milt of two males by use of the dry method (Wood and Dunn 1948). Fertilized eggs were refrigerated overnight until they could be placed into a "living stream" system (Frigid Units, Inc.), which consisted of a rectangular tank (204 × 52 × 56 cm) with a dual water chiller and pump that circulated water past the eggs, through a charcoal filter, and back to the tank via a channel under the tank's floor. The stream had a water velocity of 1–3 cm/s after being filled to 40 cm with tap water that had been "aged" for several days, allowing chlorine to dissipate.

Eggs were incubated at a mean temperature of 3.9°C (range = 2.2–5.4°C; Figure 1) under a 12 h (fluorescent) light : 12 h dark daily cycle, and dead (opaque) eggs were removed daily. From 29 d postfertilization, approximately 6% of the stream volume was exchanged with aged tap water every week to maintain water quality. When eggs developed visibly pigmented eyes (35 d postfertilization), they were transferred from the floor of the stream to three mesh nursery baskets (16 × 12 × 13 cm) floated with foam collars. Before hatching began, another "living stream" was set up and maintained in a manner similar to that of the first stream.

Each day, newly hatched larvae were transferred to an empty nursery basket located in stream 1 or 2 so that the day of hatching was known for all larvae. The temperature of each stream was increased incrementally throughout the rearing process, starting from the hatching period (Figure 1), to mimic seasonal warming after ice breakup. Additionally, stream 2 was maintained at a higher temperature than stream 1 to mimic the warmer inshore waters and embayments of Lake Superior, in contrast to colder offshore waters and upwelling regions. For this study, we used only the peak of the hatch (86%), which spanned 13 d near the end of the hatching period (Figure 1). When we observed that these larvae were ready to feed (~10 d posthatch), all larvae were fed fresh nauplii of brine shrimp *Artemia salina* once daily beginning at 106–125 d postfertilization (10–28 d posthatch) until 128 d postfertilization (29–37 d posthatch); larvae were fed twice daily thereafter. As larvae in each nursery basket continued to grow throughout the rearing period, they were divided into additional baskets to minimize crowding.

Larval growth and yolk utilization.—The overall rates of larval growth and yolk utilization under baseline laboratory conditions were determined by selecting 2–35 larvae (median = 10 larvae) at 0–75 d posthatch every 1–12 d (median = 3 d) throughout the rearing period for use in image capture; these larvae were preserved in 95% ethanol. Digital images (640 × 480 pixels) were captured from a dissecting microscope set at 1–3× magnification. From the images, we measured total length (TL), yolk length, and yolk depth using SigmaScan Pro 5 (SPSS, Inc.) and calibrated them with digital images of a stage micrometer. Larval lengths at age were used to estimate growth rates in the two streams. Because the larvae hatched over a 13-d span and because temperatures were increased periodically, larvae preserved at the same age had in some cases experienced slightly different thermal regime. Measurements of yolk length and depth were used to calculate yolk volume as a prolate spheroid: length was the longer axis, and depth and girth were the shorter and equal axes. Four larvae with exceptionally large yolk volumes were excluded from all analyses because they might have hatched prematurely. This left a total of 165 preserved larvae from stream 1 and 193 preserved larvae from stream 2.

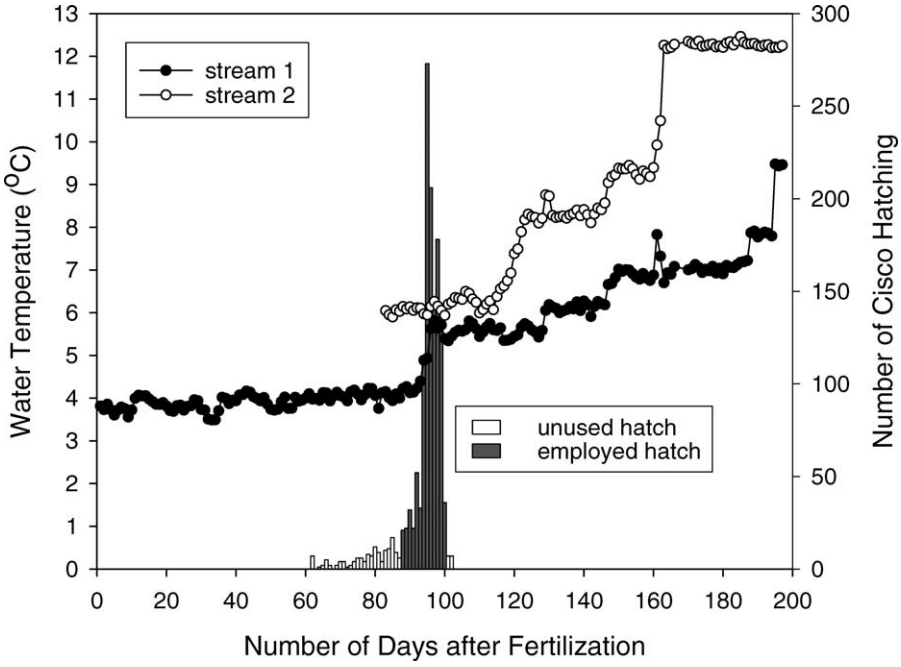

FIGURE 1.—Water temperatures and cisco hatching distribution in two laboratory streams used to rear larvae from the egg stage. Eggs were incubated in stream 1; daily hatches were segregated in either stream. Only the peak of the hatch was utilized in a study of otolith age estimation.

Otolith mounting and analysis.—We examined the otolith microstructure of selected larvae and embryos that were preserved during the rearing process by first extracting left and right sagittae with dissecting needles under a dissecting microscope equipped with a polarizing filter, which increased the reflectance of the otoliths. The otoliths were then mounted concave side down on a microscope slide with CrystalBond (SPI Supplies), a thermoplastic mounting medium.

Once mounted, individual otoliths were read blind in random order, each side separately, with a compound microscope at up to 1,000× magnification. The most consistent of three or more increment counts from each sagitta of a pair was used to calculate a mean count and SD for the larva. An index of average percent error (Beamish and Fournier 1981) was calculated as a measure of precision between the independent left and right counts. Digital images of the otoliths were captured from the compound microscope; the images were used to measure the lengths of both sagittae and of their interior regions bounded by the check mark. Mean lengths were then calculated from each pair of sagittae to represent the larva.

We mounted otoliths of 70 larvae from stream 1 and 51 larvae from stream 2, but we excluded the following from all analyses: one otolith of a yolk outlier, seven otoliths from which one side was lost during the extraction and mounting process, and seven stained or otherwise unreadable otoliths. This left 61 mounted otoliths (i.e., complete sets) from stream 1 and 45 otoliths from stream 2 in total from the otolith validation study, feeding regime experiment, and temperature shock experiment.

Otolith validation study.—We utilized 69 larvae of various ages for otolith validation from the collection of preserved larvae reared under baseline laboratory conditions; this included controls of the two experiments (19 larvae), which were also reared under baseline laboratory conditions. Including larvae from the controls ensured that they also followed normal increment deposition and thus could serve as relevant standards for comparison with the experimental treatments. Other than these control larvae, no larvae were used in more than one of the three studies.

To assess whether a check mark was deposited at hatching, five embryos were preserved during the hatching period for comparison with a subset of 17 larvae that had been preserved before first feeding. To assess whether a check mark was deposited at first feeding, the day of first feeding was established by separating larvae that fed from those that did not when food was offered for the first time. Larvae preserved

thereafter were used to assess whether increments were deposited at a daily rate. For all of these larvae, the presence or absence of yolk was used to correlate larval age and length at yolk sac absorption with otolith microstructure and possibly check formation.

Feeding regime experiment.—We assessed the effects of feeding regime on otolith deposition by subjecting 10 nursery baskets of larvae per stream to one of four treatments: twice-daily feeding (control), once-daily feeding, alternate-day feeding, or no feeding (food deprivation). These treatments occurred during 141–161 d postfertilization and lasted for 13–19 d except for the food deprivation treatment, which lasted for only 5 d, as described by Rice et al. (1985). During this period, water temperatures averaged 6.6°C (range = 5.9–7.0°C) in stream 1 and 9.0°C (range = 8.1–9.4°C) in stream 2 (Figure 1). This experiment was used to assess whether the rate of increment deposition could be altered by feeding frequency or whether larval growth reductions caused by reduced rations and lower temperatures could affect normal increment deposition. We preserved 35–63-d-old larvae at 0–14 d posttreatment and successfully mounted and read 36 pairs of otoliths. Because these larvae varied considerably in age, it was not appropriate to compare increment number directly among treatment groups. Instead, we first calculated the predicted age of each larva from increment number by use of a regression equation generated from the validation study. Predicted ages were then compared with actual ages by using the percent error equation:

$$\text{Percent error in age estimation} = \left(\frac{\text{predicted age} - \text{actual age}}{\text{actual age}}\right) \times 100,$$

where negative deviations signify cases having fewer observed increments than expected based on actual age. We used percent error as the dependent variable in the analysis to assess differences among feeding regimes and between streams.

Temperature shock experiment.—We assessed the effects of sudden temperature changes (between 6.9°C and 12.3°C) on otolith deposition by transferring larvae from one stream to the other without an acclimation period. In addition, to separate the effect of the temperature change from the effect of a new temperature, we floated larvae for approximately 30 min in their own stream within a plastic container containing water from the other stream. This allowed larvae to experience the sudden temperature change before being quickly acclimated back to their original temperature. For controls, larvae were transferred in a similar manner to a different nursery basket within the same stream. For this experiment, we calculated the width of an increment to determine recent otolith growth rates by measuring a span of five increments in the posterior region of the sagittae and then dividing that length by 5. We preserved 73–75-d-old larvae at 5–8 d posttreatment (163 or 166 d postfertilization) and successfully mounted and read 19 pairs of otoliths.

Effect of larval growth rate on the accuracy of age estimation.—Larvae from the validation study and the two experiments were combined to determine an overall relationship between larval growth rate and the accuracy in estimating age from increment number. To estimate the daily growth rate of individual larvae, we first estimated TL at hatch based on a linear regression of hatch length (TL) against hatch date (DATE) (TL = 2.24 + 0.08 • DATE; $n = 33$, $r^2 = 0.76$, $P < 0.0001$; Oyadomari 2005). The average daily growth rate was then calculated as the increase in length from hatching to preservation divided by age in days. For measuring aging accuracy, we used the percent error in age estimation as defined for the feeding regime experiment.

Larvae that were younger than 40 d were excluded from this analysis for several reasons. First, error in estimating hatch length more strongly influences the calculated daily growth rate for smaller-sized larvae. Second, the actual growth of larval fishes is typically not linear (Jones 2002), unlike what was assumed in the daily growth rate calculation; thus, younger larvae overall were expected to exhibit exceptionally lower growth rates. Finally, even a small positive or negative deviation between predicted and actual ages might cause exceptionally large variation in the percent error calculation for younger larvae, because of the fewer increments they could possibly possess. By excluding these younger larvae, we were left with 29 larvae from stream 1 and 31 larvae from stream 2 for evaluating the influence of larval growth rate on otolith deposition rate, which translates to accuracy in estimating age by otolith analysis.

Statistical analyses.—After the assumption of normality was satisfied with the Shapiro–Wilk test in the Statistical Package for the Social Sciences version 11 (SPSS, Inc.), parametric tests (*t*-test, linear regression, and two-factor analysis of variance [ANOVA]) were performed with SYSTAT 10 (SPSS, Inc.). For one case in which the data deviated significantly from a normal distribution, the Mann–Whitney test was used instead of the two-sample *t*-test. Nonlinear regressions (exponential decay for yolk utilization) were estimated with SigmaPlot version 8 (SPSS, Inc.).

Results

Larval Growth and Yolk Utilization

The growth rate of larvae increased through time in both streams as water temperature and larval size

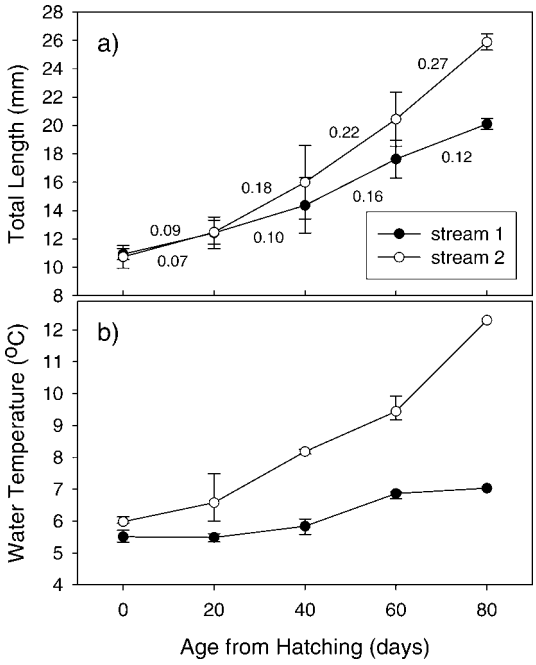

FIGURE 2.—**(a)** Overall length growth of larval ciscoes reared in two laboratory streams. Data are means (±SD) of 2–70 larvae from within 5 d of the labeled age; average daily growth rates (mm/d) are displayed between adjacent means. These data include larvae additional to those examined for otolith microstructure (n = 123 for stream 1; n = 130 for stream 2). **(b)** Mean water temperatures (±range) experienced by cisco larvae on the dates of length measurement are presented.

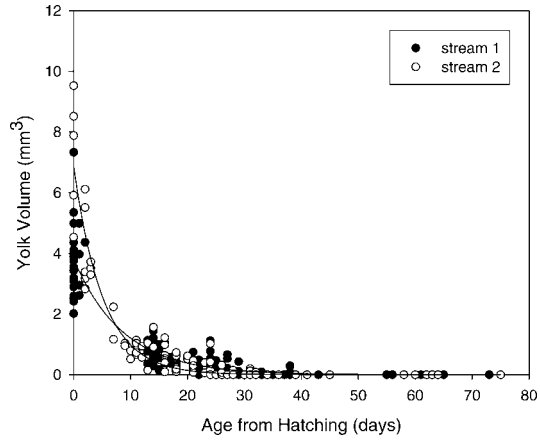

FIGURE 3.—Yolk utilization (change in yolk volume over time) by larval ciscoes reared in two laboratory streams. These data include larvae additional to those examined for otolith microstructure (n = 165 for stream 1; n = 193 for stream 2).

increased (Figure 2). However, growth in stream 2 surpassed that of stream 1 following the higher water temperature in stream 2. From a mean (±SD) hatch length of 10.69 ± 0.42 mm, larvae achieved lengths of 20.10 ± 0.40 mm in stream 1 and 25.89 ± 0.57 mm in stream 2 after 75 d. In contrast, yolk absorption followed a similar pattern of exponential decay in both streams (Figure 3). From a mean (±SD) yolk volume of 4.51 ± 2.04 mm^3 at hatch, larvae exhausted their yolks within 22–38 d. Although growth and development may have varied with hatch date and the hatch dates of these larvae spanned 13 d, mean hatch dates were similar between streams (23 March for stream 1 and 22 March for stream 2).

Otolith Increment Deposition

At hatch, otoliths had a mean (±SD) length of 65.6 ± 4.6 μm and exhibited faint and irregularly spaced increments (5.3 ± 2.0 μm) surrounding multiple primordia (Figure 4a). A comparison of otoliths from embryos, larvae at hatch, and larvae shortly after hatch but before first feeding (Figure 4b) indicated that a check mark did not form at hatch. When a check mark later formed (Figure 4c–d), the region it encircled had a length of 63.1 ± 5.6 μm, and this length did not differ statistically between streams (two-sample t-test: n_1 = 21, n_2 = 19, P = 0.50). In addition, the check length did not differ statistically with larval age (linear regression: n = 40, r^2 = 0.01, P = 0.47), indicating that the check mark was consistently identified regardless of otolith size. The check region contained faint and irregularly spaced increments averaging 5.7 ± 1.8 in number (range = 3–10), and increment number also did not differ statistically between streams (two-sample t-test: n_1 = 21, n_2 = 19, P = 0.35) or with larval age (linear regression: n = 40, r^2 = 0.004, P = 0.72). Furthermore, otoliths at hatch and at check formation did not differ statistically in increment number (two-tailed Mann–Whitney test: n_{hatch} = 8, n_{check} = 40, P = 0.35) or length (two-sample t-test: n_{hatch} = 8, n_{check} = 40, P = 0.24), suggesting that otolith growth was negligible between hatch and check formation and that the observed increments within the check mark were embryologically formed. For all larvae with a check, counts of postcheck increments between left and right sagittae had an average percent error of 8.8%.

Otolith Validation Study

Of the 69 mounted otolith pairs from larvae reared under baseline laboratory conditions, 29 did not contain a check mark. For the remaining 40 larvae, the relationship between larval age posthatch (AGE) and the number of postcheck increments (RINGS) (Figure 5) did not differ statistically between the two

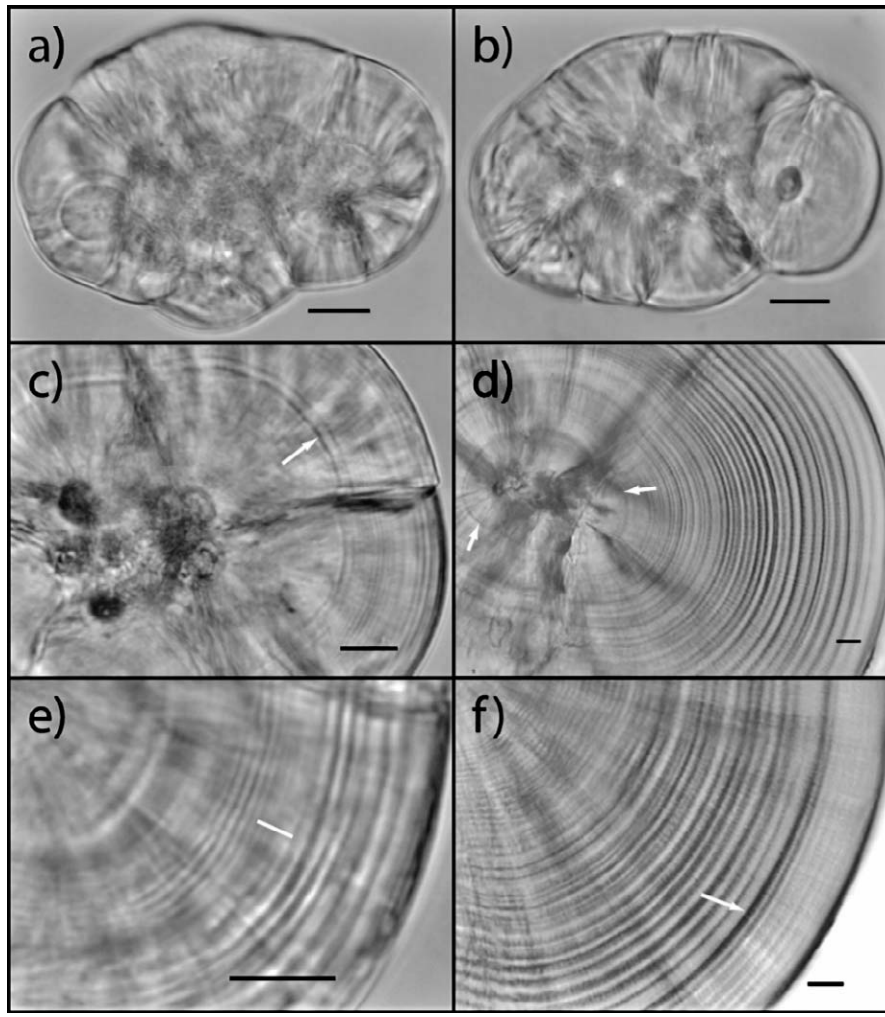

FIGURE 4.—Images of sagittae of larval ciscoes at: **(a)** hatch (no check mark); **(b)** 24 d posthatch (no check mark); **(c)** 45 d posthatch (check mark [arrow] is followed by daily increments); **(d)** 75 d posthatch (check mark [arrows] is followed by daily increments); **(e)** 63 d posthatch (showing faint and narrowing increments [white bar] corresponding to 5 d of food deprivation); and **(f)** 75 d posthatch (showing faint increments after an accessory check mark [arrow] corresponding to a sudden transfer from warmer to colder water). Scale bars represent 10 μm.

rearing streams in terms of their slopes ($P = 0.91$) or intercepts ($P = 0.31$; general linear model [GLM]: $n = 40$, $r^2 = 0.94$). Therefore, a pooled linear regression equation was estimated (AGE = 27.57 + 1.06 • RINGS; $n = 40$, $r^2 = 0.94$, $P < 0.0001$; 95% confidence interval [CI] for slope = 0.97–1.15). Increment deposition after check formation did not differ from a rate of one increment per day, as indicated by a slope that was not statistically different from 1.0 ($P = 0.19$). However, check formation did not commence at hatch but rather commenced at approximately 28 d posthatch, as indicated by the intercept ($P < 0.0001$). A linear regression using age from first feeding (FF) instead of age posthatch also estimated an intercept that was statistically greater than zero (FF = 10.97 + 1.14 • RINGS; $n = 40$, $r^2 = 0.84$, $P < 0.0001$ for an H_0 of $\beta_0 = 0$), indicating that check formation commenced after first feeding as well.

Complete yolk sac absorption is the next early life event on which the check mark may have been formed. Using yolk presence–absence data, we found a 50% likelihood of a larva exhausting its yolk by 28.94 d of age (Fieller 95% CI = 24.94– 32.74), independent of rearing stream (probit analysis: $n = 68$, $P = 0.27$). Because this age corresponded closely to the intercept age of 27.57 d, daily deposition from check formation

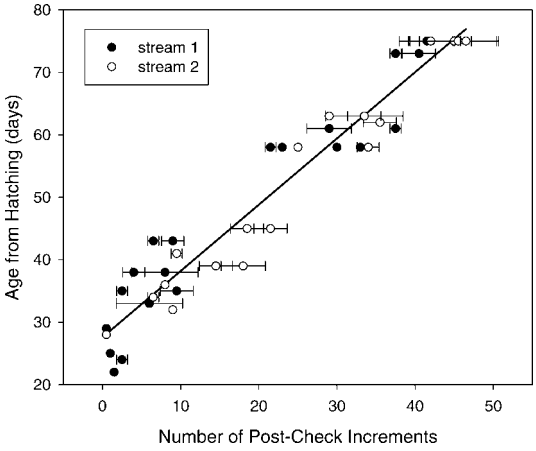

FIGURE 5.—Relationship between age posthatch and number of otolith increments exterior to the check mark in larval ciscoes reared in two laboratory streams. Data are means (±SD) of the two increment counts (left and right sagittae) for individual larvae ($n = 21$ for stream 1; $n = 19$ for stream 2).

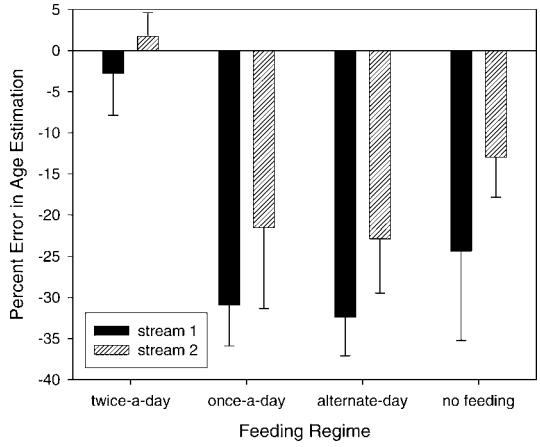

FIGURE 6.—Percent error in age estimation for larval ciscoes subjected to different feeding regimes in two laboratory streams. Negative deviations indicate that fewer increments were observed than expected. Data are means (+SE) of 3–6 larvae.

commenced near the time of yolk exhaustion. In addition, there was a close correspondence in TL when there was a 50% likelihood of larval yolk exhaustion (13.22 mm; Fieller 95% CI = 12.63–14.78) and when achieving a check mark (13.29 mm; Fieller 95% CI = 12.63–14.53).

Feeding Regime Experiment

The percent error in age estimation for 35–63-d-old larvae differed statistically among the four feeding regimes ($P = 0.0008$) but not between streams ($P = 0.08$), and the feeding regime × stream interaction was not significant ($P = 0.95$; two-factor ANOVA: $n = 36$). The three reduced-ration regimes did not differ statistically from each other (Tukey's test: $P = 0.51$ for the most significant comparison), but they each showed a significant reduction in predicted age compared with the twice-daily (control) feeding regime (Tukey's test: $P = 0.02$ for the least significant comparison). In addition, percent error in age estimation was consistently lower for stream 1 than for stream 2, but this difference was not significant (Figure 6).

Differences in feeding frequency did not affect the prominence of subdaily marks, which were faint and present only between wider increments during faster growth. However, some otoliths displayed a band of faint increments that corresponded to the 5-d period of food deprivation (Figure 4e).

Temperature Shock Experiment

Transfer of larvae from 12.3°C water in stream 2 to 6.9°C water in stream 1 (cold shock) induced an accessory check mark followed by low-contrast increments (Figure 2f). When larvae were subjected to a cold shock without remaining in the colder water thereafter, a slightly darker increment was formed but it was not easily distinguishable from the natural variation in increment contrast. Transfer of larvae from stream 1 to stream 2 (heat shock) did not induce a check mark, nor was a check mark induced in the controls. Exposure to the new water temperature for 5–8 d affected the otolith increment width of these larvae, which were 73–75 d old when the experiment concluded. Mean increment width decreased from 3.99 to 2.84 μm (paired t-test: $n = 3$, $P = 0.04$) with a decrease in temperature; increment width increased from 1.29 to 3.64 μm (paired t-test: $n = 4$, $P = 0.002$) with an increase in temperature. In contrast, the control larvae exhibited only marginal increases in increment width: from 1.18 to 1.70 μm in stream 1 (paired t-test: $n = 4$, $P = 0.02$) and from 4.07 to 5.40 μm in stream 2 (paired t-test: $n = 4$, $P = 0.05$).

Effect of Larval Growth Rate on the Accuracy of Age Estimation

Estimated growth rate varied from 0.04 to 0.25 mm/d for 40–75-d-old larvae. This variation in growth rate was attributed partly to the reduced-ration treatments. When all larvae fed twice daily were pooled together (including larvae from the temperature shock experiment), a two-factor ANOVA ($n = 60$) revealed a significant effect for feeding treatment ($P < 0.0001$) but not for stream ($P = 0.32$) or the feeding treatment × stream interaction ($P = 0.48$). Baseline feeding (twice

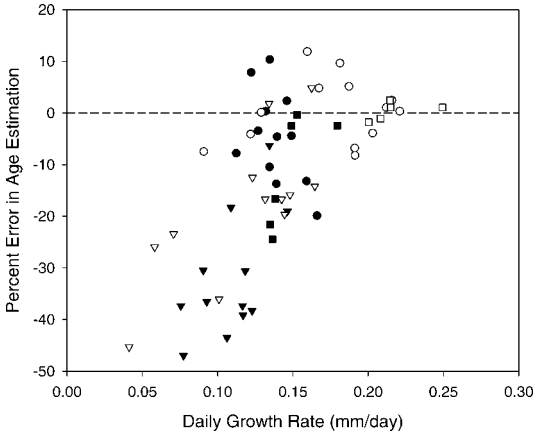

FIGURE 7.—Relationship between percent error in age estimation and daily growth rate of larval ciscoes reared in two laboratory streams (filled symbols = stream 1, $n = 29$; open symbols = stream 2, $n = 31$). Negative deviations indicate that fewer increments were observed than expected. Larvae were reared under baseline conditions (circles; including experimental controls, $n = 25$) or reduced rations (triangles; $n = 24$). Some larvae (squares; $n = 11$) were transferred across streams (these are plotted with their original stream).

daily) produced a mean growth rate that was statistically greater than those of the reduced-ration treatments (Tukey's test: $P = 0.02$ for the least significant comparison), but the reduced-ration treatments were not statistically different from each other ($P = 0.68$ for the most significant comparison). The ANOVA model, however, explained only 40% of the variation in larval growth rate. In turn, larval growth rate explained 44% of the variation in percent error associated with estimating larval age from increment number (linear regression: $n = 60$, $P < 0.0001$). Specifically, larvae with lower growth rates could not be aged as accurately, and most of them originated from the reduced-ration treatments (Figure 7).

Discussion

Otolith Validation Study

Otolith increment deposition did not differ statistically from a one increment per day rate in both streams, starting from approximately 28 d posthatch, near the time when the yolk sac was completely absorbed. However, yolk exhaustion for each individual could have occurred anytime within approximately a 15-d span. The timing of yolk exhaustion was not determined individually because of the potential stress associated with removing larvae from water repeatedly for microscopic examinations. Such stress might have caused artificial patterns in otolith microstructure. Accordingly, variation in the timing of yolk exhaustion among individuals probably contributed to some of the variation in the relationship between increment number and age. It appeared that the rate of yolk utilization was unaffected by the temperature difference between rearing streams or by experimentally delaying exogenous feeding (Oyadomari 2005). In addition, Kowalchyk (1996) found that ciscoes from Keweenaw Bay in Lake Superior depleted their yolks at a size similar to that we observed in the laboratory.

In contrast, the greatest variation in the timing of yolk exhaustion occurred among larvae of different hatch dates. The earliest-hatching larvae (which were excluded from this study) were estimated to require approximately twice the duration to utilize their yolk reserves (Oyadomari 2005). However, these larvae probably hatched prematurely, as they carried larger yolk sacs (mean = 11.74 versus 4.51 mm^3) and did not swim until a later date, at which time newly hatched larvae swam readily. John and Hasler (1956) similarly observed 6 weeks of minimal premature hatching before 3–4 d of bulk hatching for ciscoes incubated in hatchery jars with natural water pumped from Lake Mendota, Wisconsin. Whether natural populations follow the same hatching pattern is not known, but if they do, prematurely hatched larvae would constitute only a small fraction of the annual cohort. Additionally, John and Hasler (1956) concluded that hatching did not occur under the ice, and Cucin and Faber (1985) first encountered cisco larvae 3–5 d after ice breakup in Lake Opeongo, Ontario. Accordingly, increased illumination, water temperatures, and wave-induced agitation (see John and Hasler 1956) associated with ice breakup probably trigger natural hatching to occur over a brief duration. Therefore, the slight variation in time to yolk exhaustion should have only a limited influence on aging accuracy of wild-caught larval ciscoes.

The onset of daily deposition in ciscoes was later than in most other *Coregonus* spp. (Rice et al. 1985; Eckmann and Rey 1987; Klink and Eckmann 1992; Huuskonen et al. 1998). Klink and Eckmann (1992) showed that the onset of daily deposition in European whitefish can be delayed by low temperatures. Our initial rearing temperatures were approximately 1°C lower than that of Rice et al. (1985) for Lake Michigan bloaters, but they were comparable to Lake Superior spring temperatures (typically 3–7°C) in which ciscoes had been initially caught (Hatch and Underhill 1984; Oyadomari and Auer 2004). It might have been possible to induce an earlier onset of daily deposition by subjecting cisco larvae to higher temperatures than what they experienced in this study or what they normally experience in Lake Superior. For example, by acclimating European whitefish larvae to 12°C at

hatch, Eckmann and Rey (1987) found that daily deposition started with a check mark at hatch. However, wild-caught European whitefish larvae did not exhibit daily deposition until a few weeks posthatch, when growth accelerated with increasing temperatures (Eckmann and Pusch 1989; Rey and Eckmann 1989). Rey and Eckmann (1989) also noted that European whitefish larvae in the laboratory did not exhibit daily increments when reared at 4°C or 6°C (compared with 8°C or above), but hatching in nature occurs at water temperatures of 4°C or less. Because our study strived to mimic conditions that larval ciscoes typically experience in nature, the results are probably more representative of the otolith microstructure of wild-caught larvae than if laboratory conditions were set to optimize larval growth at higher, but unnatural, temperatures.

It was also possible that feeding rations influenced the onset of daily deposition. The ciscoes used for otolith analysis began feeding at 14–19 d posthatch; the additional larvae used only for estimation of growth and yolk utilization rates began feeding as early as 10 d posthatch. This first feeding age is typical for ciscoes in nature (Pritchard 1930; Savino and Hudson 1995), although feeding may begin as early as at hatch (John and Hasler 1956; Selgeby et al. 1994). We offered some larvae food on the day of hatching and at 3 d posthatch but they did not feed. Thus, we do not know what the earliest feeding date would have been if larvae were offered food daily from hatching or how a slightly earlier onset of feeding might affect the onset of daily deposition. However, once the larvae began to feed, they were given enough food—with some remaining in the rearing stream—that their guts always contained food between feedings. Observed growth rates were also comparable with those reported by Rice et al. (1985) for laboratory-reared bloaters. Therefore, the delayed onset of daily deposition in the laboratory was probably not a result of unnatural or substandard feeding conditions.

Feeding Regime Experiment

Increased feeding frequency has been known to increase increment number slightly (Neilson and Geen 1982), probably by increasing the occurrence of subdaily marks, which may confound daily counts (Campana 1983; Campana and Neilson 1985). In this study, the prominence of subdaily marks was not affected by feeding frequency. They were faint in ciscoes and visible only between wider increments during faster growth. Eckmann and Rey (1987) found that subdaily marks in European whitefish and sandfelchen were also easily discernible from daily increments. In contrast, subdaily marks occurring during faster growth confounded daily deposition in vendace (Huuskonen and Karjalainen 1995) but probably because of lower calcium concentrations in Finnish lakes (Huuskonen et al. 1998).

Larvae from the reduced-ration treatments (once-daily feeding, alternate-day feeding, and food deprivation) exhibited fewer increments than larvae from the control (twice daily) feeding treatment. There was also a tendency (although not significant) for aging accuracy to be further reduced when the limited rations were coupled with the colder rearing temperature. These factors may have (1) reduced increment width below that discernible with light microscopy, (2) delayed the onset of daily deposition, or (3) suppressed the deposition rate of subsequent increments when daily deposition had already commenced. Klink and Eckmann (1992) found that reduced temperatures (compared with 8°C) delayed the onset of daily deposition in European whitefish by 9–18 d at 6°C and by 17–30 d at 4°C; the delay was greater when reduced temperature was coupled with limited feeding (once daily compared with every 2 h). In comparison, Marshall and Parker (1982) found that daily increment deposition in sockeye salmon *Oncorhynchus nerka* ceased because of reduced temperatures (<5°C compared with ~12°C); after normal temperatures resumed, recovery to a daily rate was further impeded when a 3-week period of starvation was coupled with the reduced temperatures. The narrowing of increments with a 5-d period of food deprivation indicated that daily deposition in ciscoes can be obscured by a poor feeding condition. In addition, there was a nonsignificant tendency for daily deposition to commence approximately 3 d later in stream 1 than in stream 2, but after increment deposition commenced the deposition rate was nearly identical between streams. Therefore, fewer increments may have been produced under reduced food rations and lower water temperatures because of both a slight delay in the onset of daily deposition and an interruption of daily deposition.

Temperature Shock Experiment

A sudden temperature transition from warm (12.3°C) to cold (7.1°C) water, but not the reverse, induced a check mark in ciscoes such that subsequent increments were narrower and of lower contrast. Likewise, Eckmann and Rey (1987) found that a cold shock from 12.5°C to 4.5°C induced a check mark in 60% of European whitefish and sandfelchen, followed by narrower increments. Additional accessory check marks were also observed in ciscoes but only in a small percentage (16%) of otoliths examined. Accessory check marks did not appear to occur in response to any particular event, and even if they did, only a small

percentage of larvae deposited them. Until it is better understood what causes accessory check marks to form in ciscoes, the marks will be of only limited value as markers of environmental conditions. However, drastic changes in increment width may prove useful as markers of temperature transitions created by currents, upwellings, or migrations.

Effect of Larval Growth Rate on the Accuracy of Age Estimation

Limited rations caused by a period of reduced feeding frequency resulted in reduced larval growth rates, and lower growth rates appeared to decrease the accuracy in age estimation. Specifically, the rate of increment deposition deteriorated as growth dropped below 0.15 mm/d for 40–75-d-old larvae, particularly those given a reduced-ration feeding regime. Larval growth rate, especially when inhibited by food availability, apparently limits the degree to which otolith microstructural analysis can be used to accurately age larval ciscoes. However, larval growth in the laboratory was slower than observations in nature based on catch data. For Lake Superior, Hatch and Underhill (1988) estimated a growth rate of 0.69 mm/d (standard length) for the first week posthatch in the Duluth–Superior area, but they believed this was an overestimation. For St Mary's River, Jude et al. (1998) estimated growth rates of 0.02–1.35 mm/d (mean = 0.27 mm/d) for 8–25-mm larvae from late April to late May, and data presented by Savino et al. (1994) showed that mean length increased from 11.6 to 14.6 mm between 5 and 20 May, which translates to a mean growth rate of 0.2 mm/d. For Oneida Lake (New York), Clady (1976) presented 6 years of mean length data (range = 11.8–20.6 mm) at capture dates ranging from 8 May to 3 June, which translate to growth rates of 0.37–1.03 mm/d (mean = 0.63 mm/d). For the Bay of Quinte (Lake Ontario), Pritchard (1930) presented mean lengths (range = 10.1–17.5 mm) for daily captures from 9 May to 1 June (except for a 5-d break), which translated into an overall growth rate of 0.38 mm/d (linear regression: $n = 19$, $r^2 = 0.95$, $P < 0.0001$). Therefore, growth rates of larval ciscoes will probably not normally be low enough in nature to inhibit the accuracy in age estimation with otolith analysis. In addition, applying the regression equation from the validation study to larvae captured from Lake Superior off the western coast of the Keweenaw Peninsula, we estimated that hatching occurred primarily between mid-April and early May (Oyadomari 2005), which corresponds to previous reports based on catch data from western Lake Superior (Hatch and Underhill 1988) and the St. Mary's River (Jude et al. 1998). However, adverse growth conditions may still occur in circumstances of exceptionally low food availability, low water temperatures, or both. Therefore, we caution against the use of otolith analysis for larval ciscoes unless it can be established that environmental conditions are suitable for fostering faster growth or until aging accuracy can be improved for slower-growing larvae.

In conclusion, otolith analysis can be used to age larval ciscoes from approximately the time they absorb their yolks. The accuracy of aging, however, is dependent on larval growth rates. Further studies using electron microscopy will be needed to determine if daily deposition ceases at reduced growth rates (e.g., Klink and Eckmann 1992) or if daily increments continue to be deposited but are just too narrow to be discernable with light microscopy (Campana and Neilson 1985). If daily increments are still deposited, electron microscopy could greatly improve estimates of larval age. Otolith increment patterns may also prove useful in tracking environmental changes that larvae experience (e.g., Rice et al. 1987; Eckmann and Pusch 1989). For ciscoes in particular, shifts in increment width and contrast may signify shifts in temperature or feeding success, and the formation of accessory check marks may signify sudden decreases in temperature. However, further studies are needed to determine the extent of a temperature decrease necessary to induce a check mark and to determine whether other factors can also induce accessory check marks. In general, otolith analysis has the potential to improve our understanding of the dynamics of early life survival and recruitment in ciscoes by providing a method that can be used to track larval movements with age distributions and increment patterns and to determine the favorability of different habitats with estimated larval growth rates.

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